```
PDK1       84 FGKILGEGSFSTVVLARELATSREYAIKILEKRHIIKENKVPYVTRERDVMSRLD-HPFFVKLYFTFQDDEKLYFGLSYAK 163
DSTPK61   164 FGRYLGEGSYSIVYLAVDIHSRREYAIKVCEKRLILRERKQDYIKRERDVMHQMTNVPGFVNLSCTFQDQRSLYFVMTYAR 244
Pkh1      127 FGEQLGDGSYSSVVLATARDSGKKYAVKVLSKEYLIRQKKVKYVTVEKLALQKLNGHKGIFKLFFTFQDEASLYFLLEYAP 207
Pkh2      181 FGSVIGDGAYSTVMLATSIDTKKRYAAKVLNKEYLIRQKKVKYVSIEKTALQKLNNSPSVVRLFSTFQDESSLYFLLEYAP 261

PDK1      164 NGELLKYIRKIGSFDETCTRFYTAEIVSALEYLHGKGIIHRDLKPENILLNEDMHIQITDFGTAKVLS------------ 231
DSTPK61   245 KGDMLPYINRVGSFDVACTRHYAAELLLACEHMHRRNVVHRDLKPENILLDEDMHTLIADFGSAKVMTAHERALATEHCSE 325
Pkh1      208 HGDFLGLIKKYGSLNETCARYYAAQIIDAVDSLHNIGIIHRDIKPENILLDKNMKVKLTDFGTAKILPE------------ 276
Pkh2      262 NGDFLSLMKKYGSLDETCARYYAAQIIDAIDYLHSNGIIHRDIKPENILLDGEMKIKLTDFGTAKLLN------------ 329

PDK1      232 ------------------------------------------------PESKQ-----ARANSFVGTAQYVSPELLTEKSACKS 262
DSTPK61   326 QRRSNSDEDDEDSDRLENEDEDFYDRDSEELDDRDDEQQQEEMDSPRHRQRRYNRHRKASFVGTAQYVSPEVLQNGPITPA 406
Pkh1      277 ------------------------------------------EPSNTADGKPYFDLYARSKSFVGTAEYVSPELLNDNYTDSR 317
Pkh2      330 ------------------------------------------PTNNSVSKPEYDLSTRSKSFVGTAEYVSPELLNDSFTDYR 369

PDK1      263 SDLWALGCIIYQLVAGLPPFRAGNEYLIFQKIIKLEYDFPEKFFPKARDLVEKLLVLDATKRLGCEEMEGYGP-IKAHPF 341
DSTPK61   407 ADLWALGCIVYQMIAGLPPFRGSNDYVIFKEILDCAVDFPQGFDKDAEDLVRKLLRVDPRDRLGAQDEFGYYESIRAHPF 486
Pkh1      318 CDIWAFGCILYQMLAGKPPFKAANEYLTFQKVMKIQYAFTAGFPQIVKDLVKKLLVRDPNDRL-------TIKQIKAHLF 390
Pkh2      370 CDIWAFGCILFQMIAGKPPFKATNEYLTFQKVMKVQYAFTPGFPLIIRDLVKKILVKNLDRRL-------TISQIKEHKF 442
```

Fig. 1a

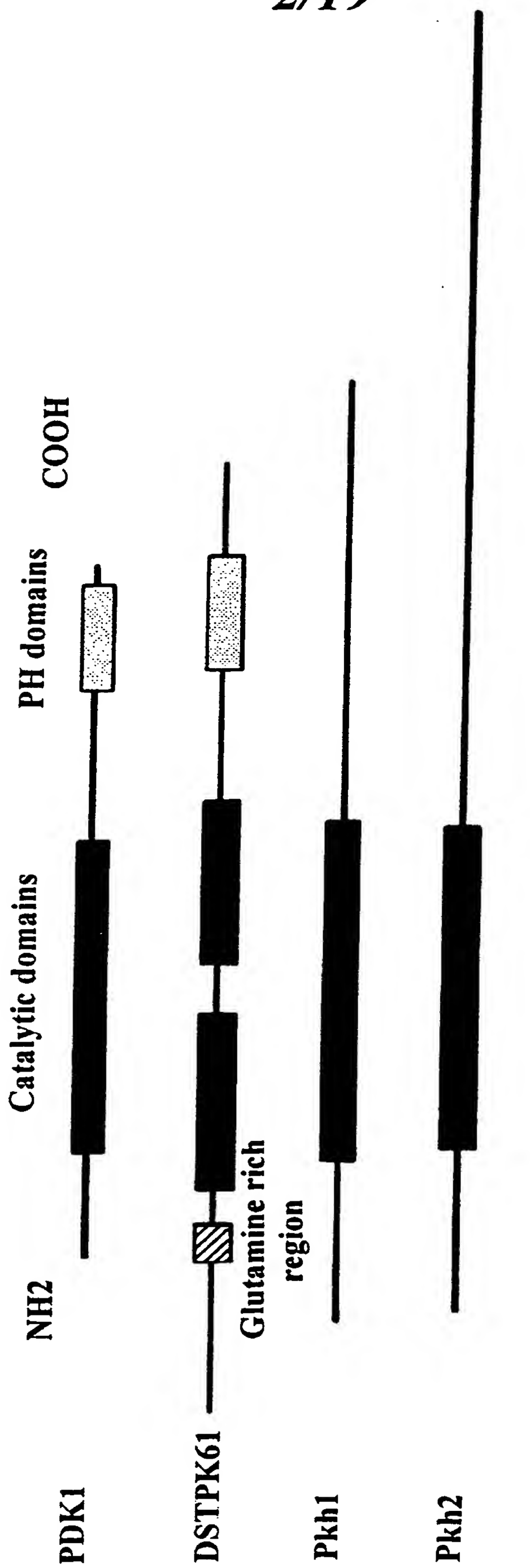
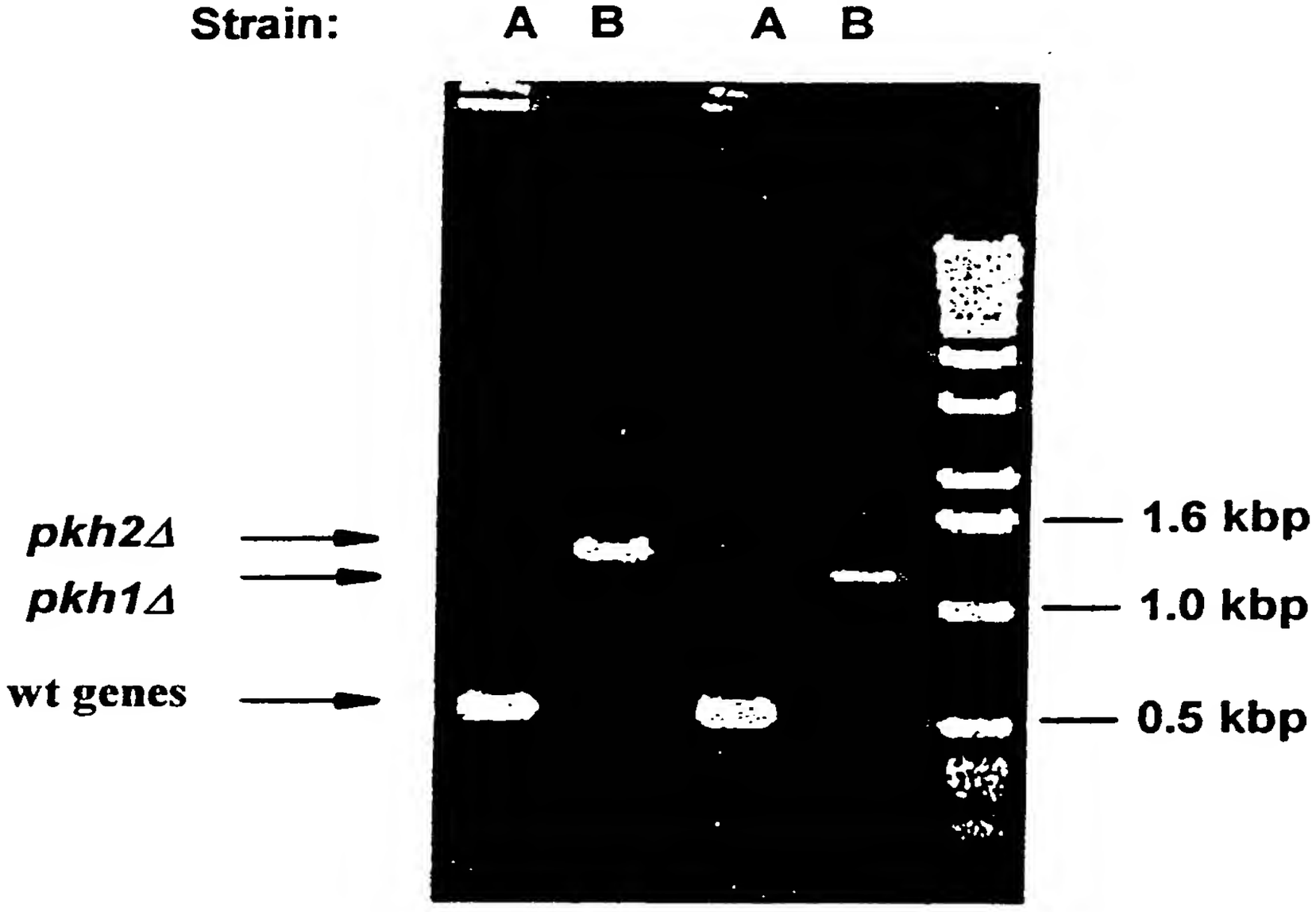

NH2
Catalytic domains
PH domains
COOH
Glutamine rich region
PDK1
DSTPK61
Pkh1
Pkh2

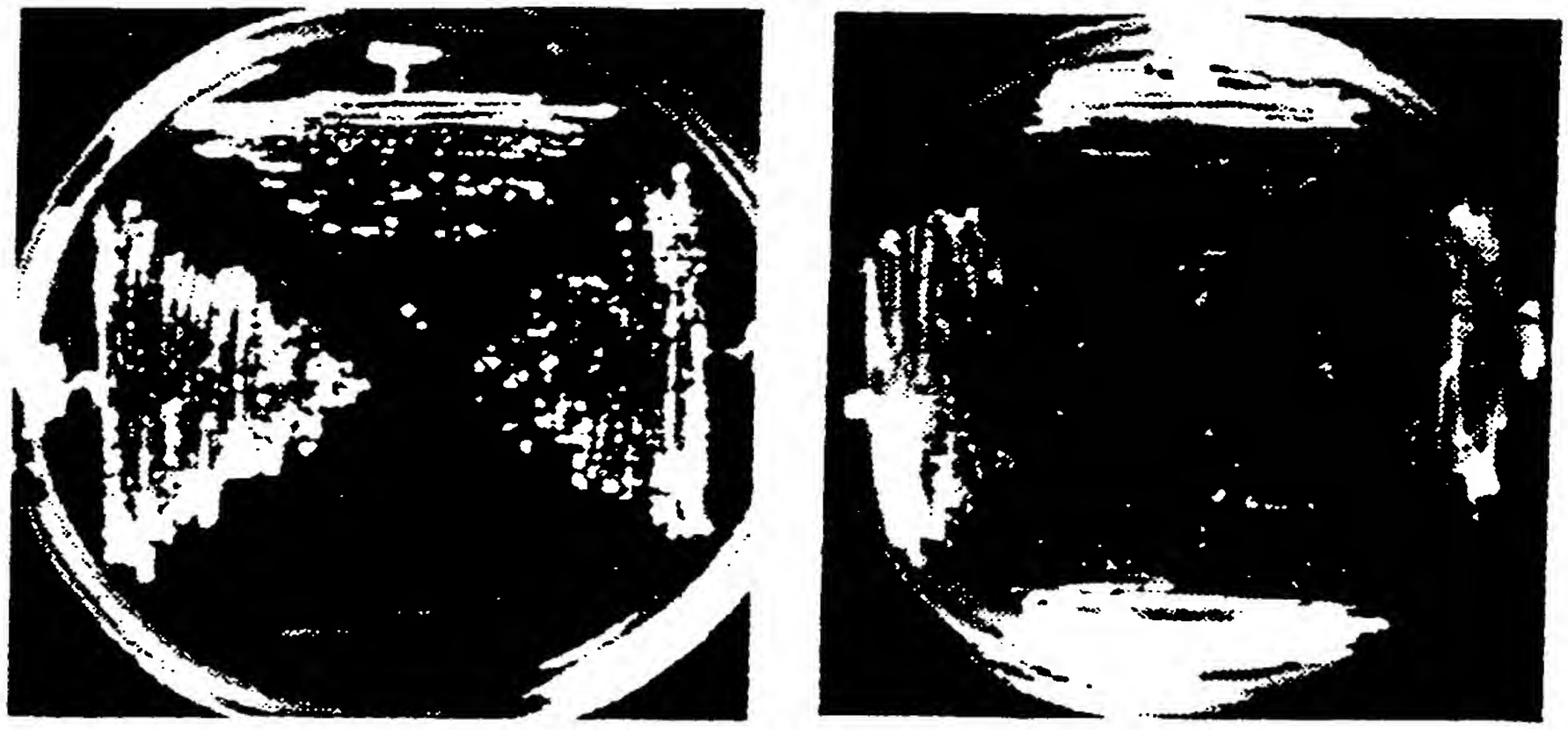
Fig. 1b

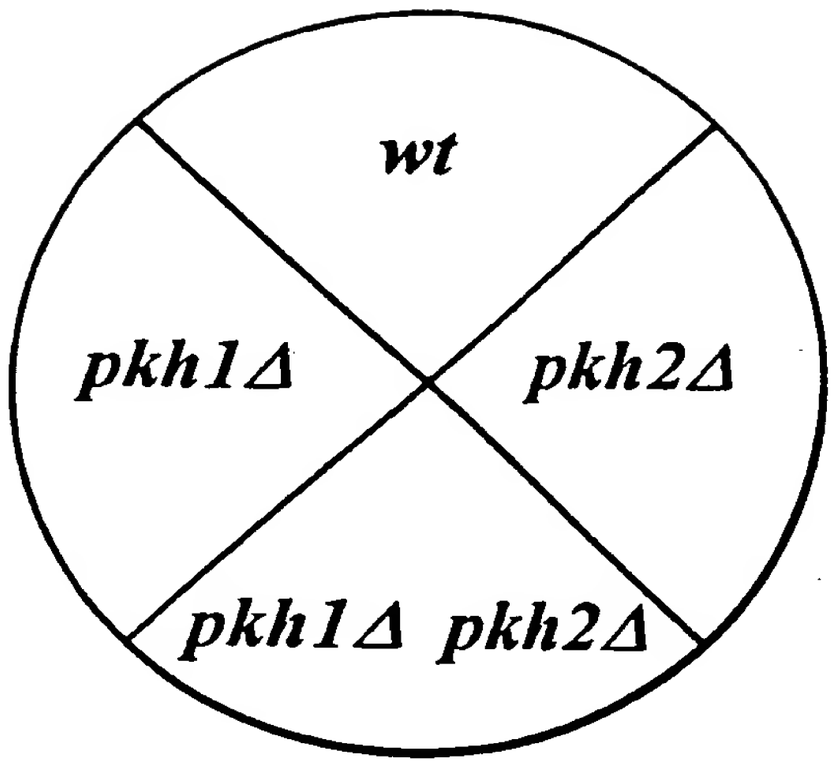


Strain A: wt [pYES2-*PKH1*]

Strain B: *pkh1Δ pkh2Δ* [pYES2-*PKH1*]

Fig. 2A





+pYES2-*PKH1*

Fig. 2B

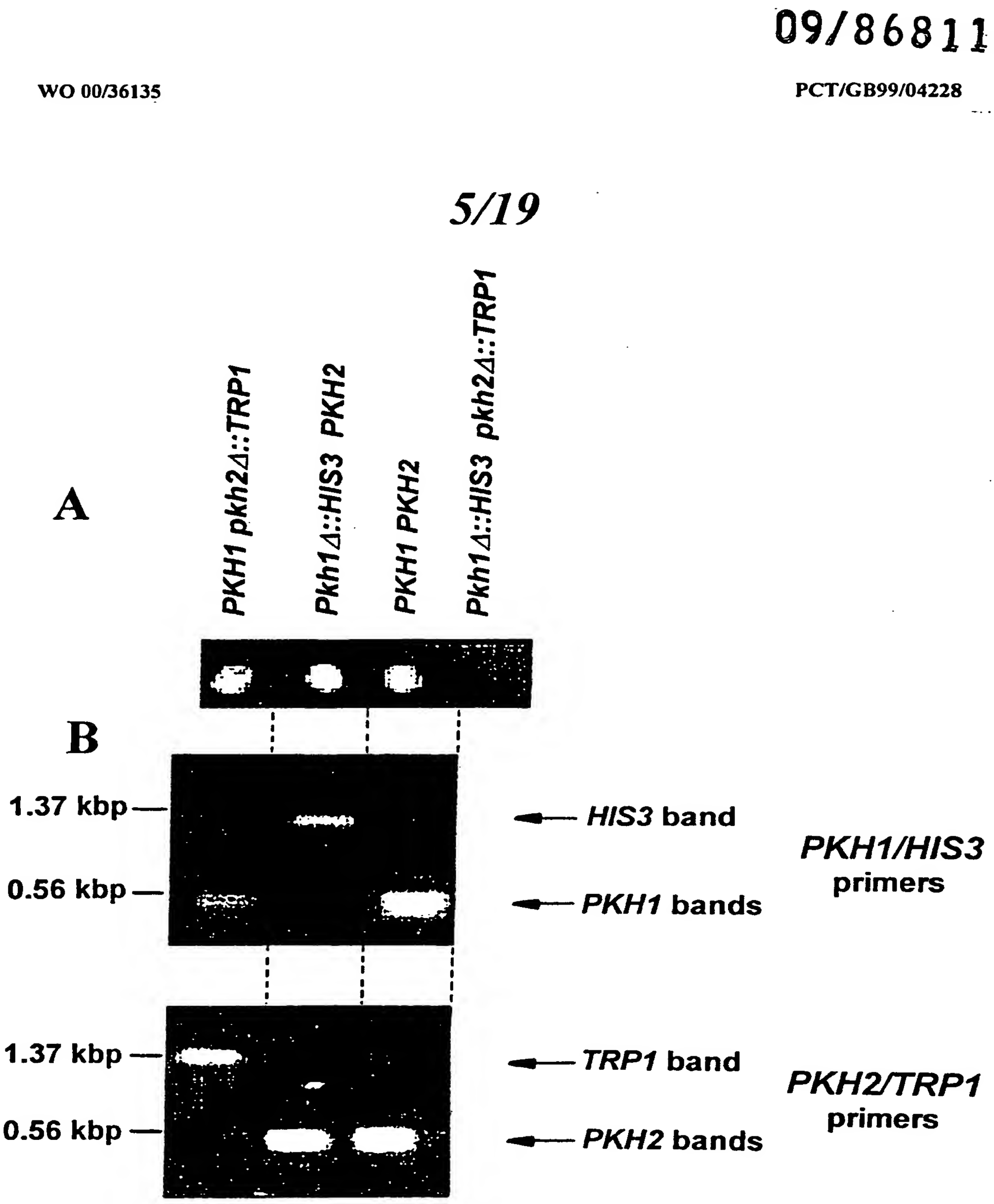
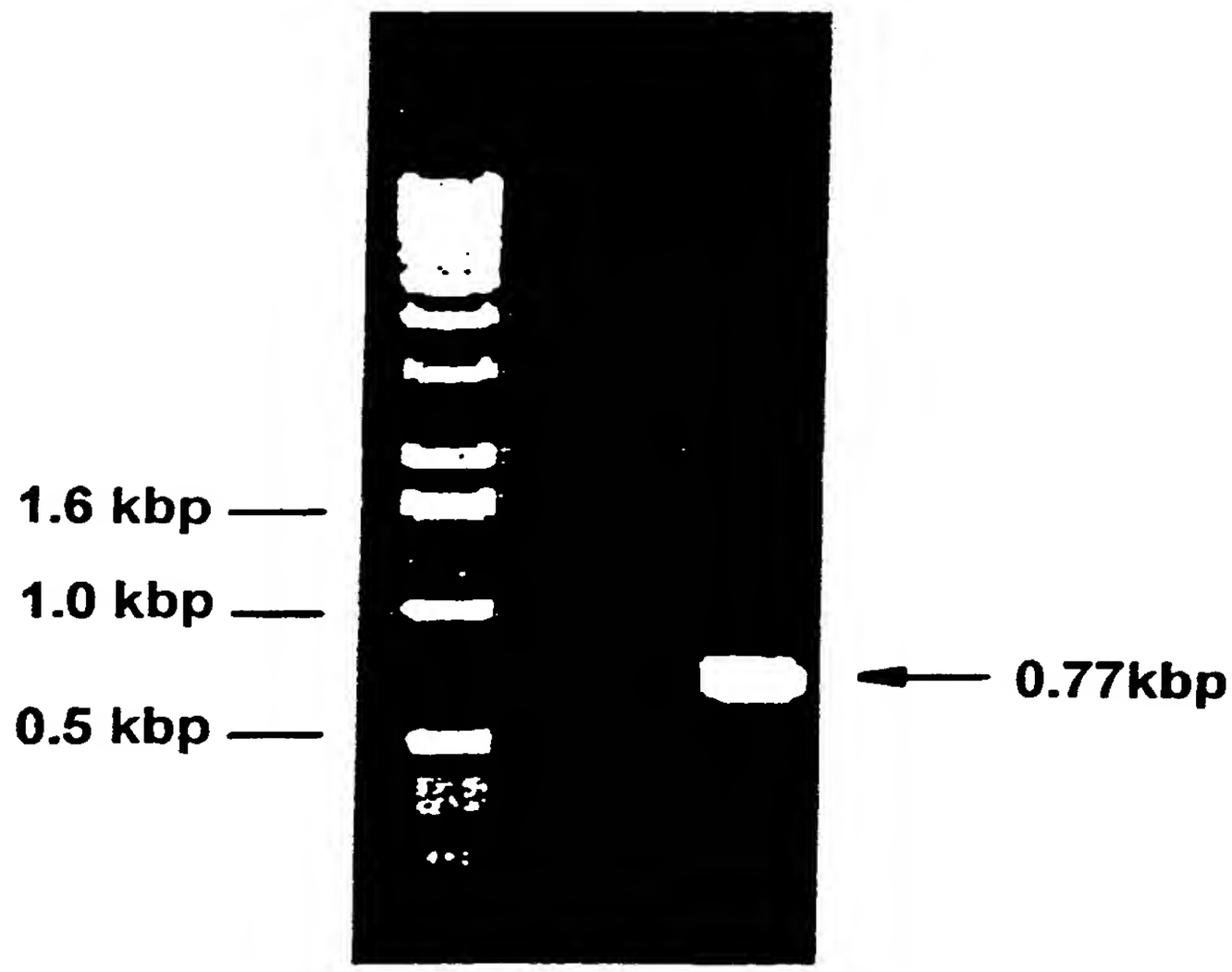
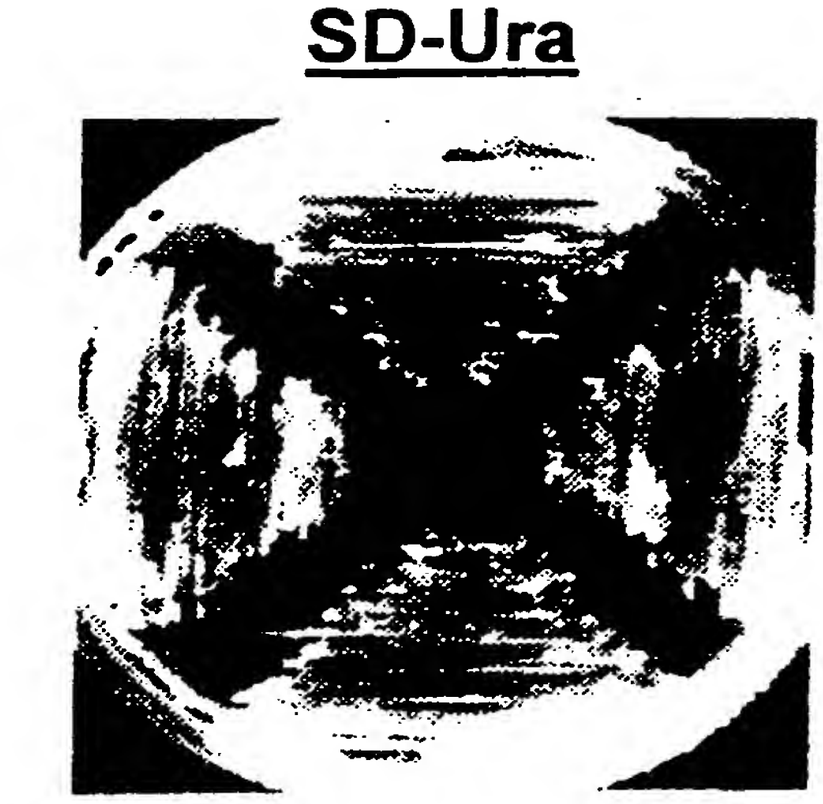

A
PKH1 pkh2Δ::TRP1
Pkh1Δ::HIS3 PKH2
PKH1 PKH2
Pkh1Δ::HIS3 pkh2Δ::TRP1
B
1.37 kbp
0.56 kbp
HIS3 band
PKH1 bands
PKH1/HIS3 primers
1.37 kbp
0.56 kbp
TRP1 band
PKH2 bands
PKH2/TRP1 primers

Fig. 3

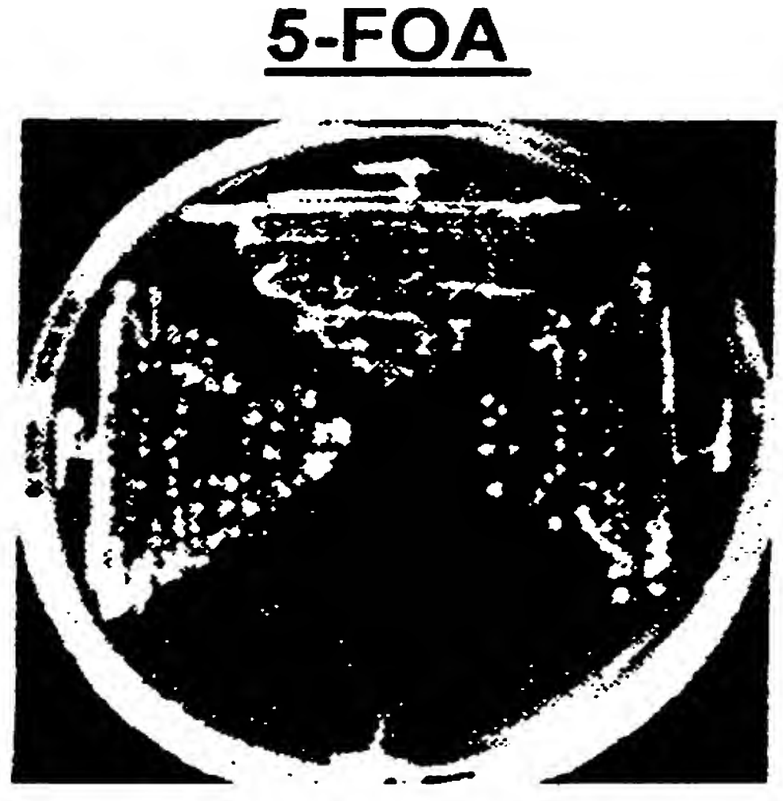


Strain A: *pkh1Δ pkh2Δ* [YEplac195-*PKH1*]
Strain B: *pkh1Δ pkh2Δ* [YEplac195-PDK1]

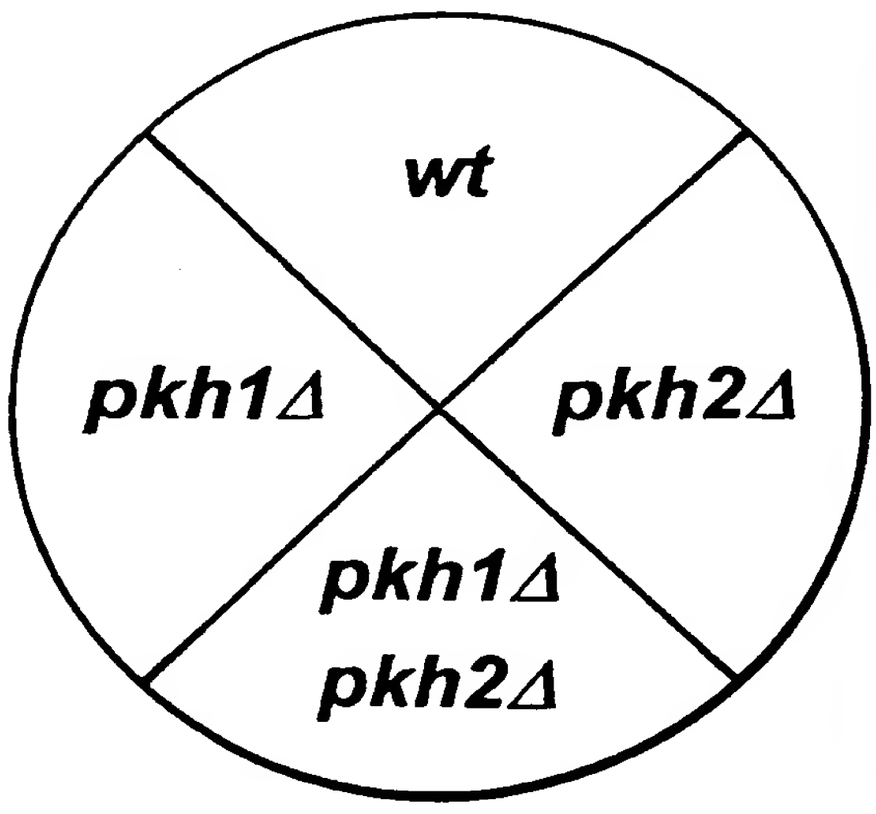
Fig. 4A

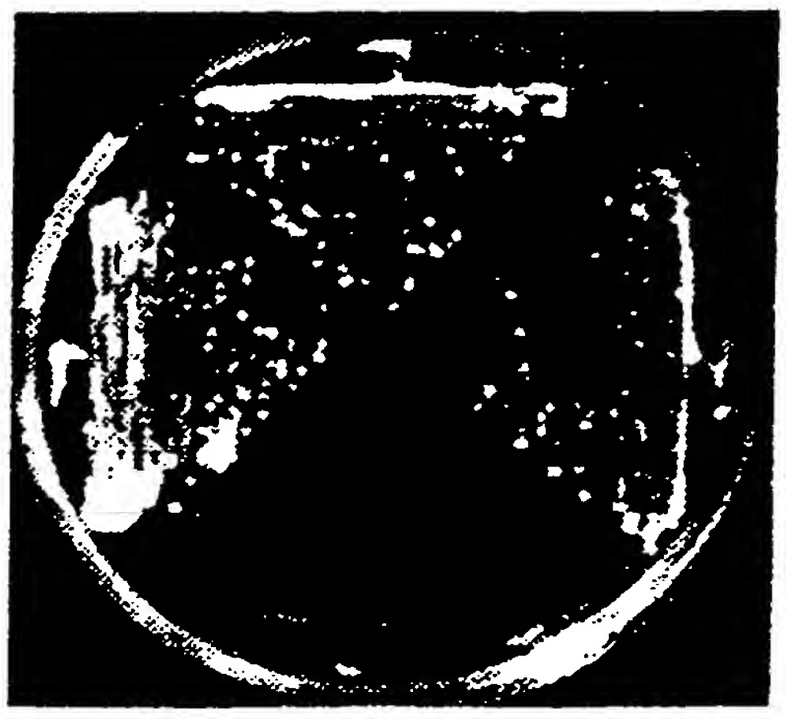


[YEplac195-PDK1]
or
[YEplac195-ΔPH-PDK1]



[YEplac195-PDK1]





[YEplac195-DPH-PDK1]

Fig. 4B

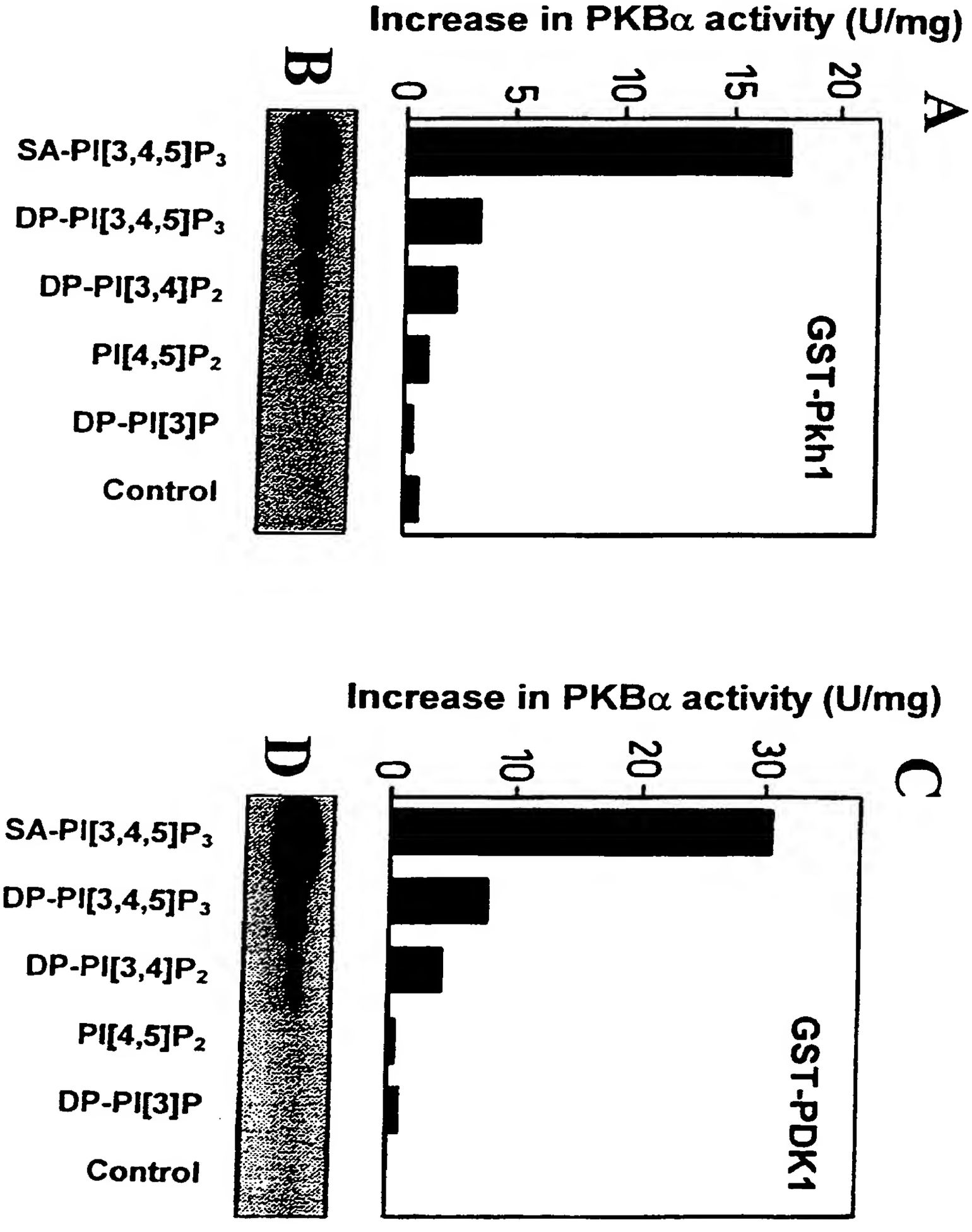


Fig. 5

WO 00/36135
PCT/GB99/04228
09/868118

Human enzymes	Residue	Amino Acid Sequences	Residue
PKBα	303	GATMK**T**FCGTPEYLAPE.....FPQF**S**YSAS	477
SGK	251	NSTTS**T**FCGTPEYLAPE.....FLGF**S**YAPP	426
p70S6Kα	224	GTVTH**T**FCGTIEYMAPE.....FLGF**T**YVAP	393
PKCζ	533	DAKTN**T**FCGTPDYIAPE.....FRNF**S**FMNP	699
Yeast enzymes			
Ypk1	499	DDKTD**T**FCGTPEYLAPE.....FGGW**T**YVG-	665
Ykr2	496	NDKTD**T**FCGTPEYLAPE.....FGGW**T**YIG-	662
Pkc1	978	GNRTS**T**FCGTPEFMAPE.....FRGF**S**FMPD	1147
Sch9	565	KDRTN**T**FCGTTEYLAPE.....FAGF**T**FVDE	741

PDK1 phosphorylation site

PDK2 phosphorylation site

Fig. 6A

09/868118

PCT/GB99/04228

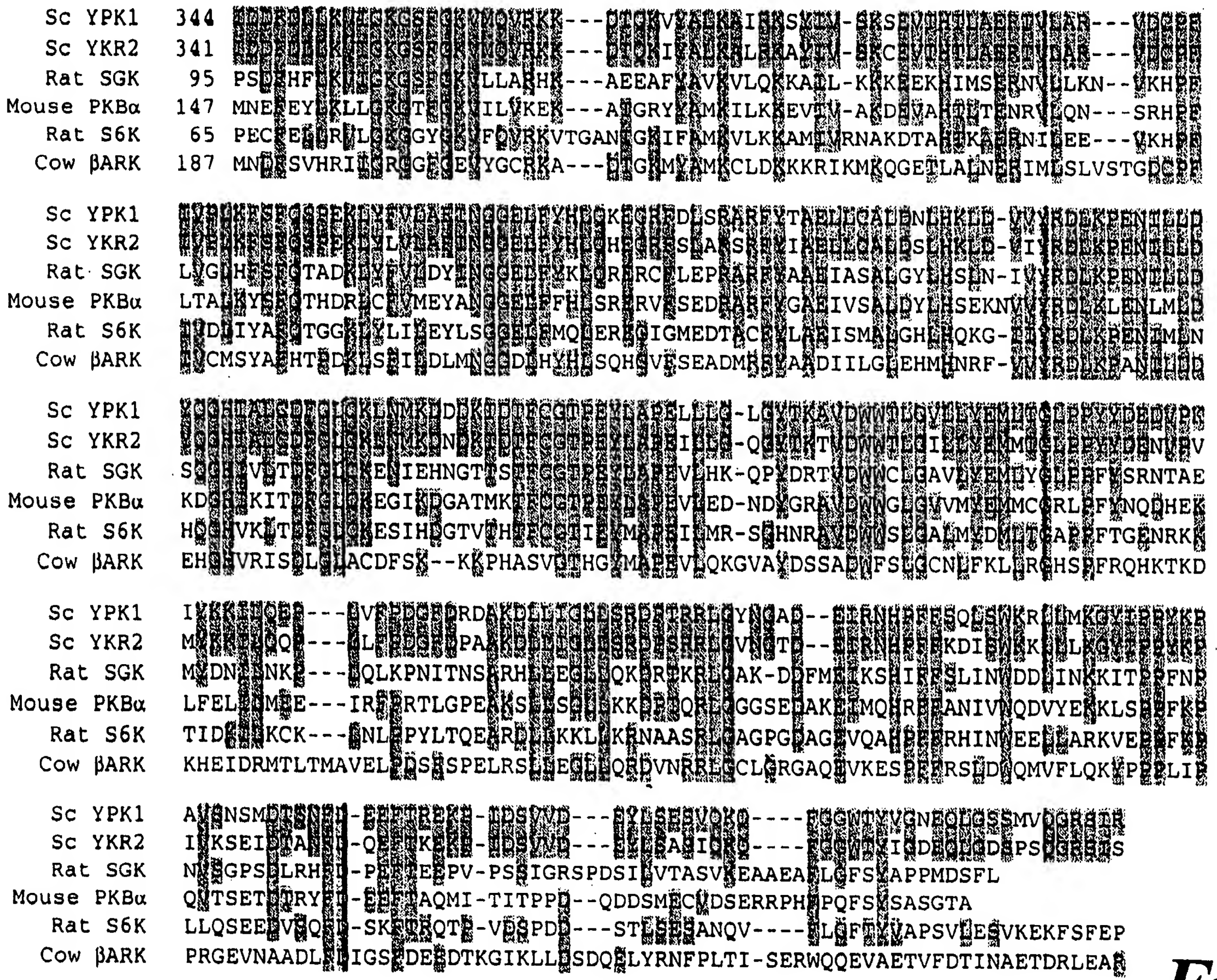

Sc YPK1 344
Sc YKR2 341
Rat SGK 95
Mouse PKBα 147
Rat S6K 65
Cow βARK 187
Sc YPK1
Sc YKR2
Rat SGK
Mouse PKBα
Rat S6K
Cow βARK
Sc YPK1
Sc YKR2
Rat SGK
Mouse PKBα
Rat S6K
Cow βARK
Sc YPK1
Sc YKR2
Rat SGK
Mouse PKBα
Rat S6K
Cow βARK

Fig. 6B

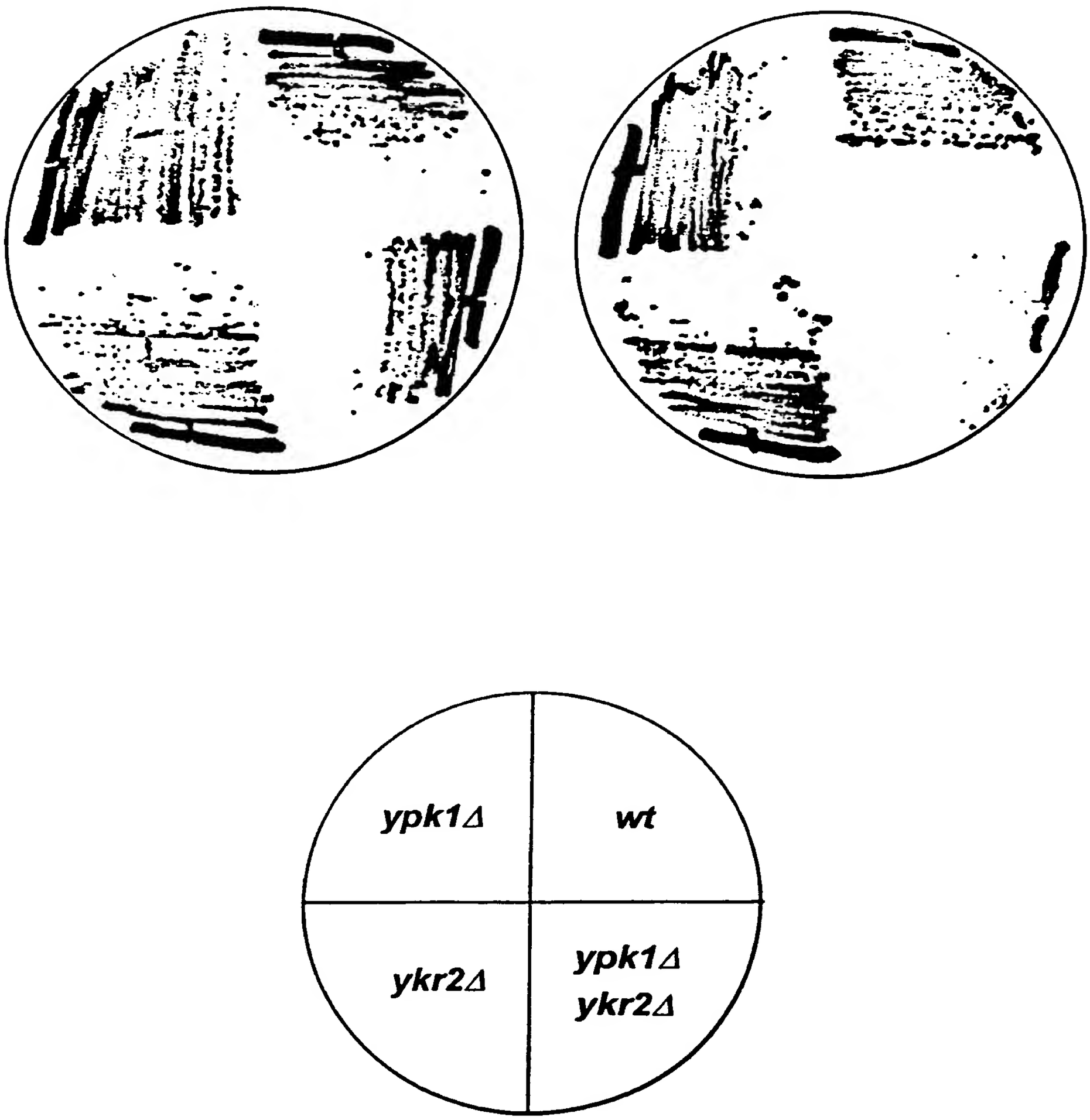


Fig. 7

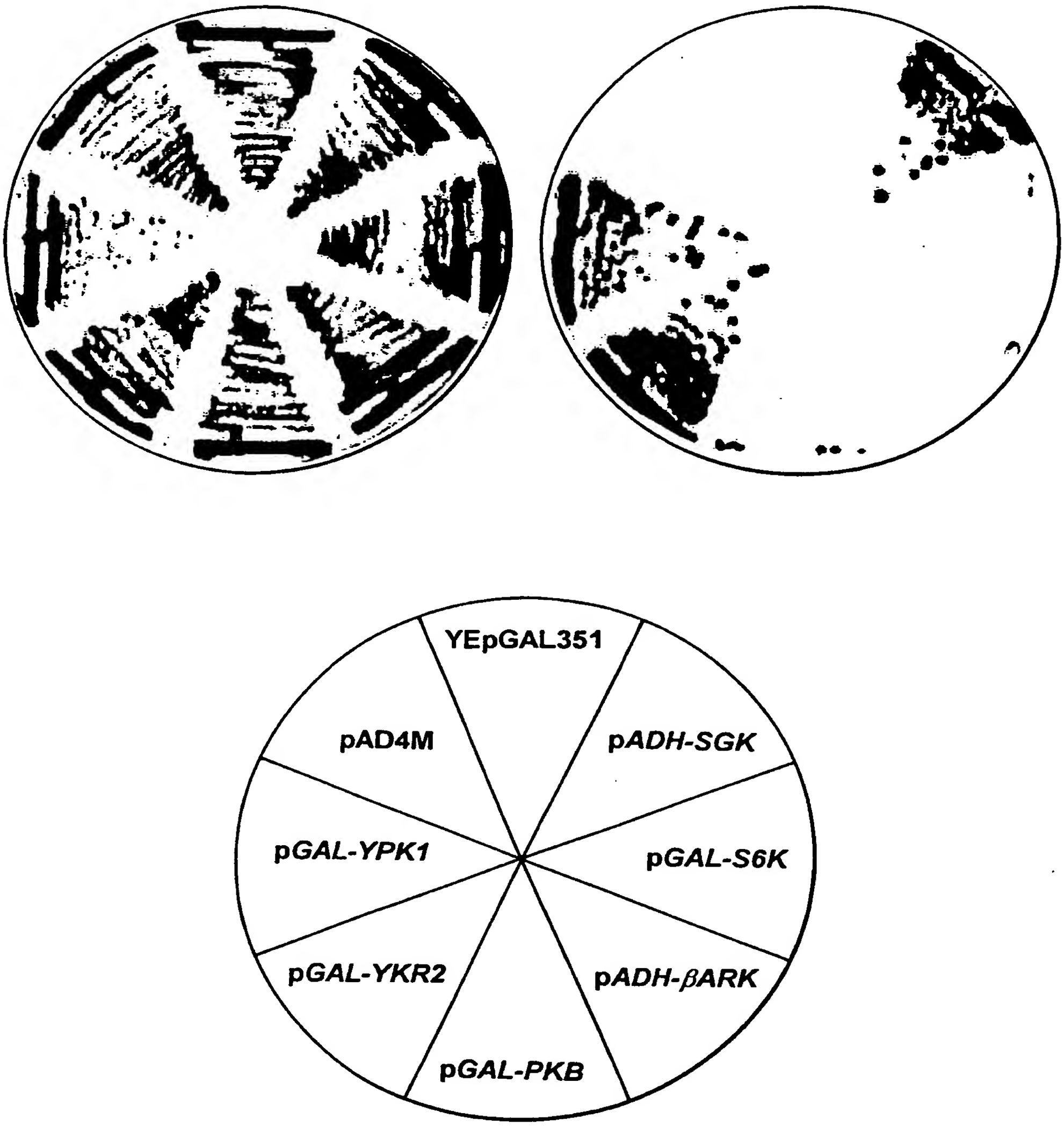

SCGal-Leu
+ 5-FOA
YEpGAL351
pAD4M
pADH-SGK
pGAL-YPK1
pGAL-S6K
pGAL-YKR2
pADH-βARK
pGAL-PKB
ypk1Δ ykr2Δ [pYKR2 (URA3)]

Fig. 8A

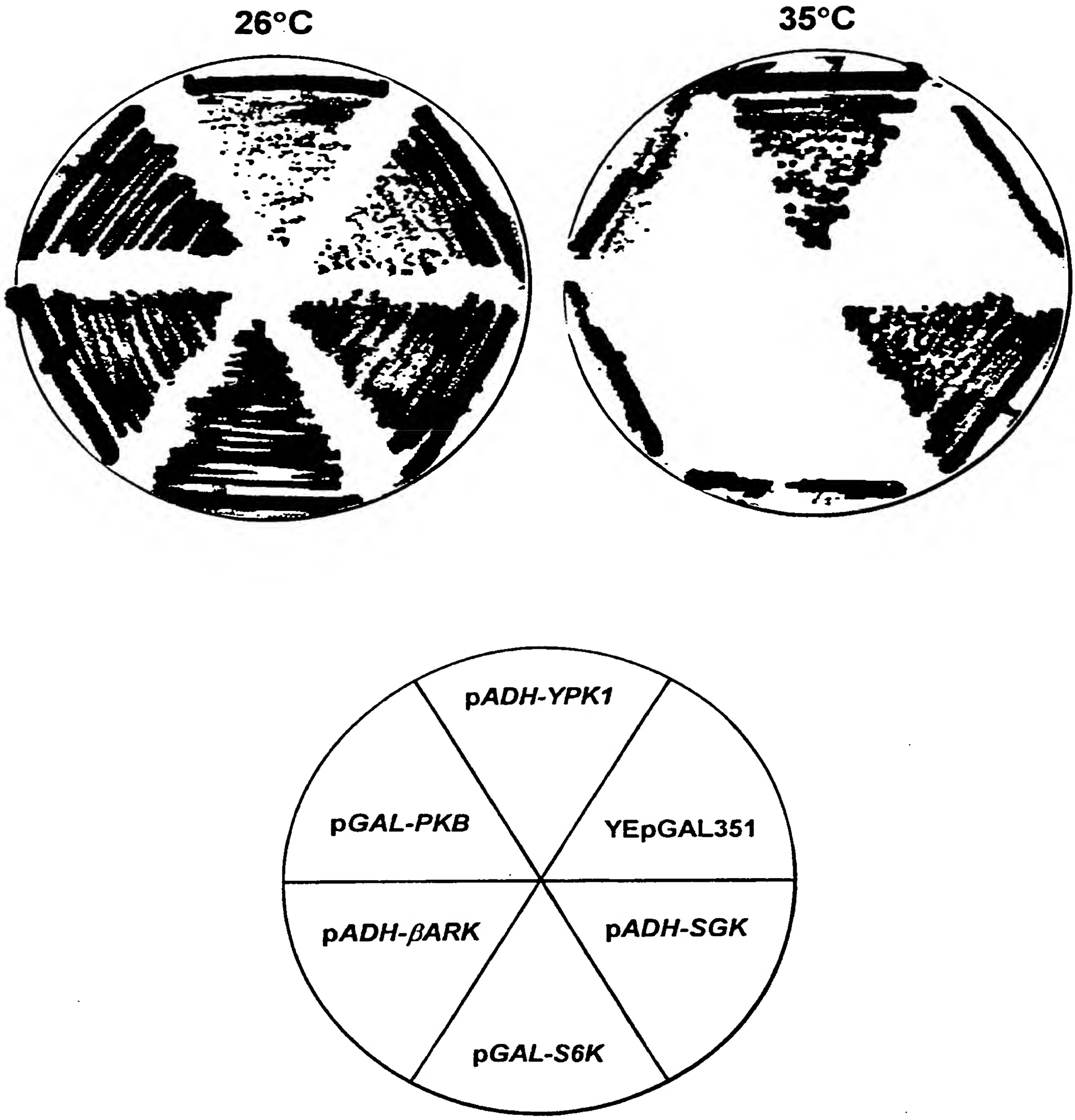

26°C
35°C
pADH-YPK1
pGAL-PKB
YEpGAL351
pADH-βARK
pADH-SGK
pGAL-S6K
ypkk1-1 ykr2Δ

Fig. 8B

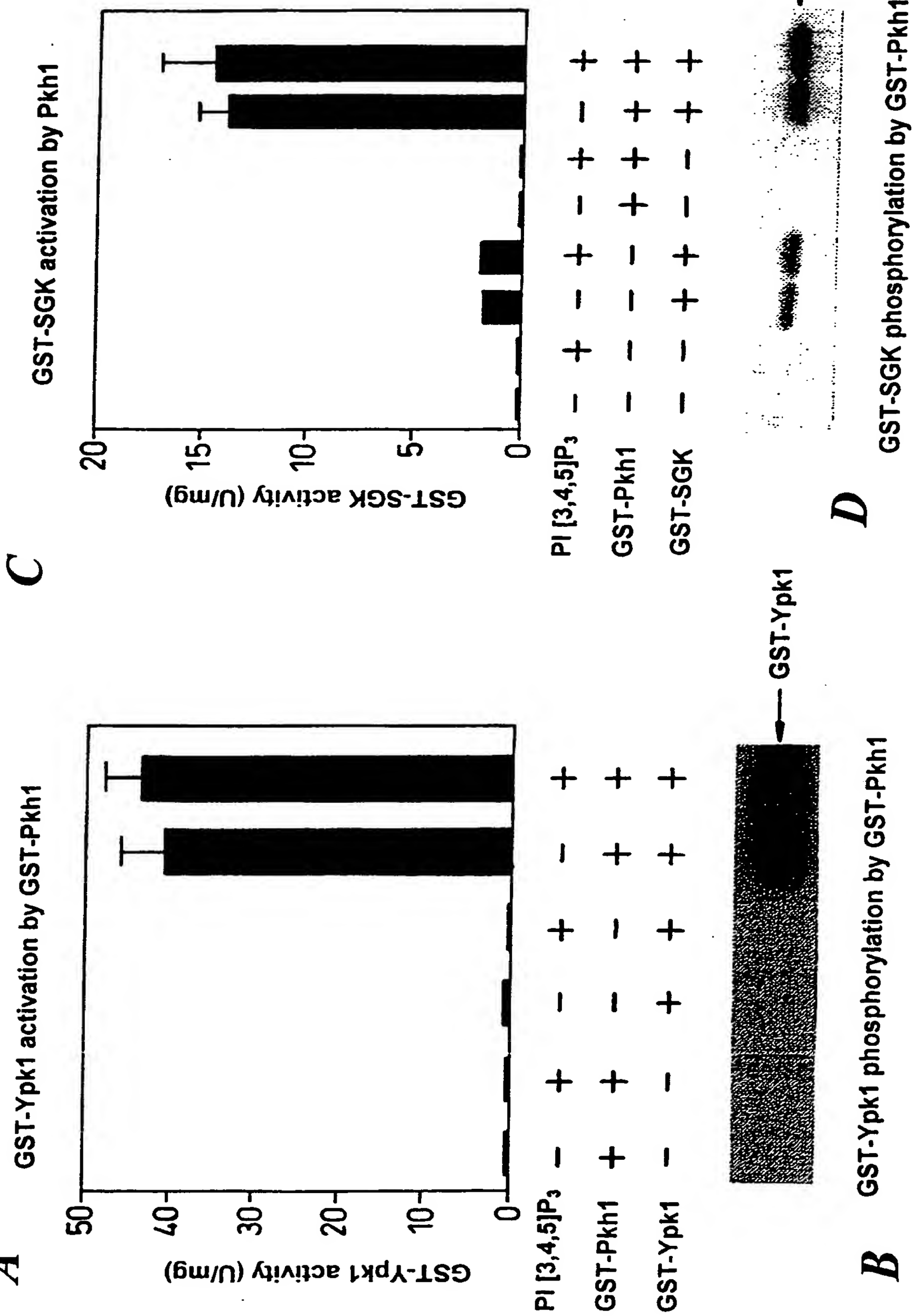
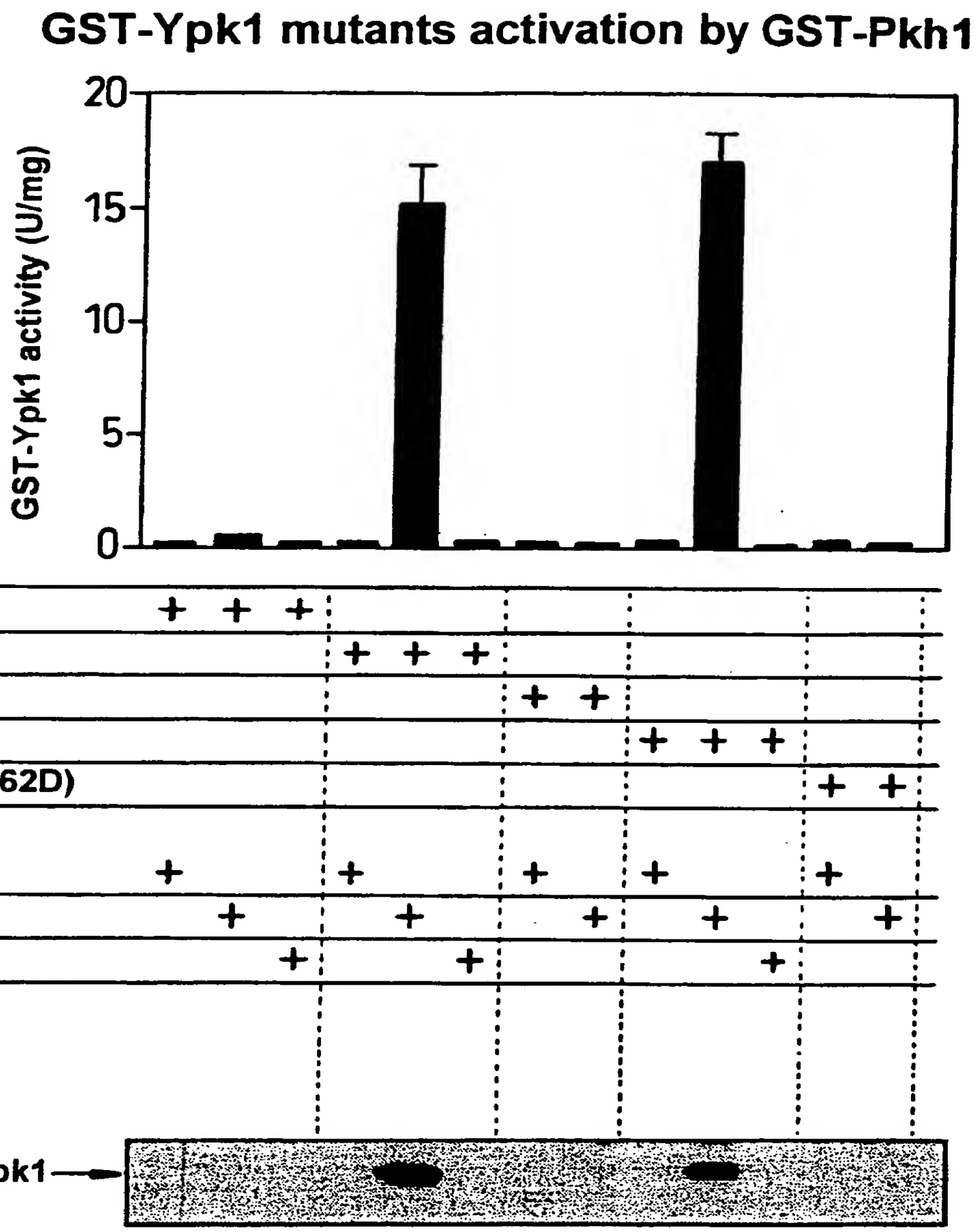

A
GST-Ypk1 activation by GST-Pkh1
GST-Ypk1 activity (U/mg)
50
40
30
20
10
0
PI [3,4,5]P3 − + − +
GST-Pkh1 + + − −
GST-Ypk1 − − + +
B
GST-Ypk1 phosphorylation by GST-Pkh1
GST-Ypk1
C
GST-SGK activation by GST-Pkh1
GST-SGK activity (U/mg)
20
15
10
5
0
PI [3,4,5]P3 − + − + − +
GST-Pkh1 − − + + − −
GST-SGK − − − − + +
D
GST-SGK phosphorylation by GST-Pkh1
GST-SGK

Fig. 9

A



B

GST-Ypk1 mutants phosphorylation by GST-Pkh1

Fig. 10

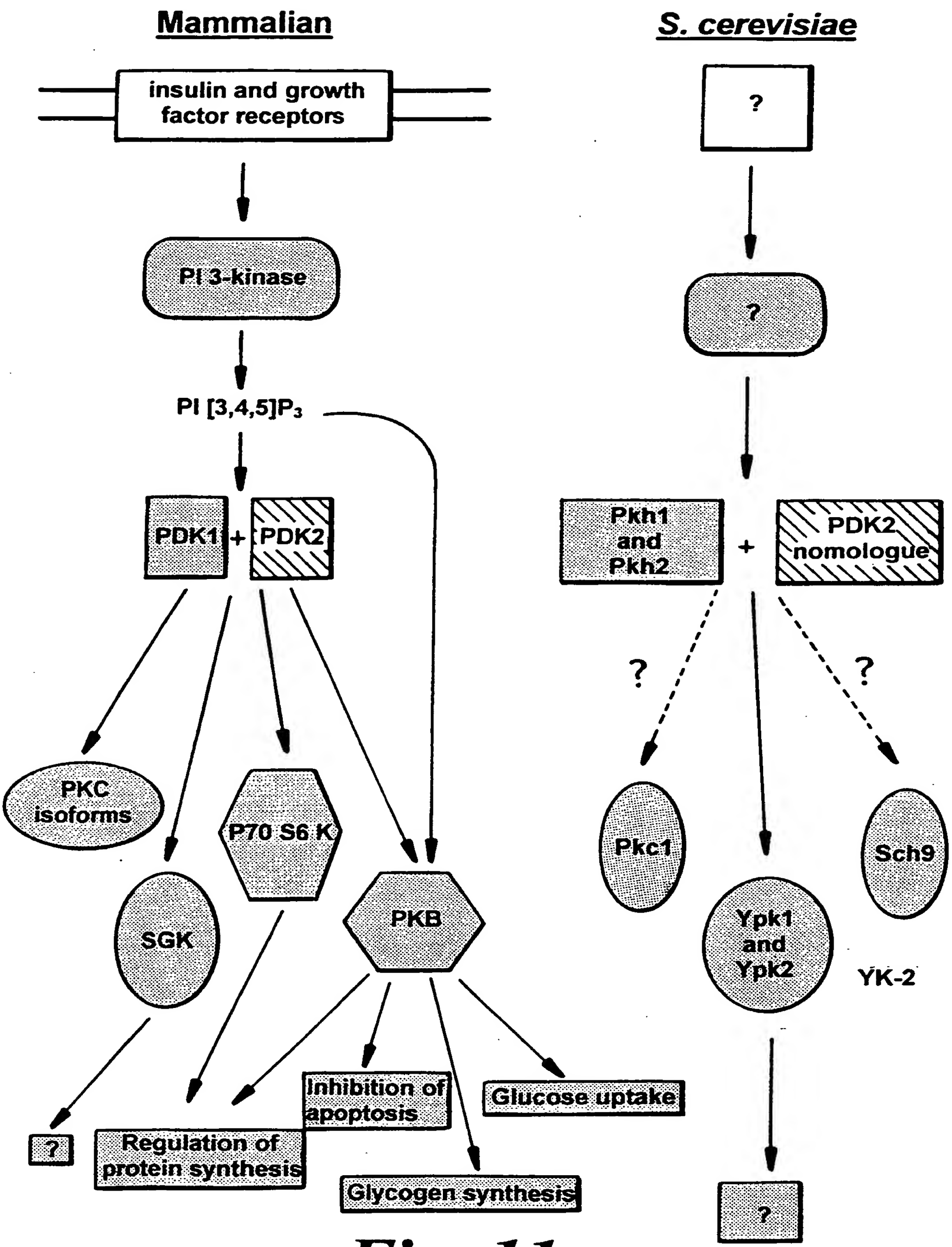

Mammalian
S. cerevisiae
insulin and growth factor receptors
?
PI 3-kinase
?
PI [3,4,5]P3
PDK1
+
PDK2
Pkh1 and Pkh2
+
PDK2 nomologue
PKC isoforms
P70 S6 K
PKB
SGK
?
Regulation of protein synthesis
Inhibition of apoptosis
Glycogen synthesis
Glucose uptake
?
?
Pkc1
Sch9
Ypk1 and Ypk2
YK-2
?

Fig. 11

CGTCAAAACCGAGGCTGCTCGAAGTACCCTCACCTACTCCAGAATGAGGGGAAT
GGTAGCAATCCTCATCGCTTTCATGAAACAGAGAAGGATGGGCCTGAACGATTT
TATTCAGAAGCTTGCCAACAACTCCTATGCATGCAAACACCCTGAAGTTCAATC
CTATTTGAAAATCTCCCAACCTCAGGAGCCCGAACTTATGAACGCCAACCCCTC
ACCTCCTCCAAGTCCCTCTCAACAAATCAACCTGGGTCCATCCTCAAATCCCCA
CGCCAAACCCTCTGACTTCCACTTCTTGAAAGTGATCGGAAAAGGCAGTTTTGG
AAAGGTTCTTCTAGCAAGGCACAAGGCAGAAGAAGCATTCTATGCCGTCAAAGT
TTTGCAGAAGAAAGCCATCTTGAAGAAGAAGGAGGAGAAGCATATTATGTCAGA
GCGCAATGTTCTGTTGAAGAATGTGAAGCACCCTTTCCTGGTGGGCCTTCACTT
CTCTTTCCAGACTGCTGACAAACTCTACTTCGTCCTAGACTACATTAATGGCGG
AGAGCTGTTCTACCATCTCCAGAGGGAGCGCTGCTTCCTGGAACCCCGTGCTCG
CTTCTACGCAGCTGAAATAGCCAGTGCCTTGGGTTATCTGCACTCCCTAAACAT
CGTTTATCGAGACTTAAAACCAGAGAATATTCTCCTAGACTCACAGGGACACAT
CGTCCTCACTGACTTTGGGCTCTGCAAGGAGAACATCGAGCACAATGGGACAAC
GTCCACCTTCTGTGGCACGCCTGAGTATCTCGCTCCTGAGGTTCTCCATAAGCA
GCCGTACGACCGGACAGTGGACTGGTGGTGCCTCGGGGCTGTCTTGTATGAGAT
GCTCTATGGCCTGCCTCCGTTCTACAGCCGGAACACAGCCGAGATGTATGACAA
TATTCTGAACAAGCCTCTCCAGCTGAAAAATATCACCAACTCAGCAAGGCACCT
GCTGGAGGGCCTCCTGCAGAAGGACCGGACCAAGAGGCTGGGTGCCAAGGATGA
CTTTATGGAGATTAAGAGTCATATTTTCTTCTCTTTGATTAACTGGGATGATCT
CATTAATAAGAAGATCACGCCCCCATTTAACCCAAATGTGAGCGGGCCCAGTGA
CCTTCGGCACTTTGATCCCGAGTTTACTGAGGAGCCGGTCCCCAGCTCCATCGG
GCGATCCCCTGACAGCATCCTTGTCACAGCCAGTGTGAAAGAAGCCGCGGAAGC
CTTCCTTGGCTTCTCCTATGCCCCTCCTATGGACTCCTTCCTCTGAAAGCTCCC
AGGATGGTTCCGAAGGATTTCCTCAGCGTTTTTCTAAAGTGTTTTAGTTAGCCT
TTGGTGGAGTTACCAGCTGACAGAACATCTTAGAAGAGAAATTTGCACACCAGG
AAGCTTGGCAGTCCCGCCTGCCGGGGCCTGCACGCGGCTTGTTGACGCGGAAGC
TTTCCGGAAGCTTTCCGAAGAGCACATCCTCCTCTCAGTGAGCTAGTGAGGTCT
TCATTTCTTTTCTTCCTTCCAACGTGGTGCTAGCTCTAAAGGAGCTTGAGAGTG
CCGCCTGAGACGCACCTTGGTCTCAGTGAGAAGGAAGATGCAGGTCTAAGAGGG
ATCTCCGCAGGTCTGAGCTGTGATCAAGAATATTCTGCAATGTGCCTTTTCTGA

Fig. 12 (part 1 of 2)

GATTGTGTTAGCTCCAAAGCTTTTCCTATCGCAGAGTGTCCAGTTTTTGTTTGT
TTTTTTTTTTTTGTTTTGTTTTTTGTTTCTTTTTTTTTCCCAACCCTTGCGTATT
TCCCATGTGTGCAGTTAGTGTGAGTGCTATGCCTGATCACAGACAGTTTTGTTG
TAAGCATCAATGTGACACTTGCAGGACACTACAATGTGGGACATTGTTTGTTTC
TTCCACATTTGGAAGATAAATTTATGCGCAGACTGTTTTTTTTTTGTAAGATATA
ATAACTAAAACCTATTGAAACGGTCTTGCAGTGACGAGCATCCAGATGCTTGAG
GGAAGCATTGCTGCTACAAATATTTCTATTTTTAGAAAGGGTTTTTATGGACCA
ATGCCCCAGTTGTCAGTCAGAGCCGTTGGTGTTCATGTTAAAATGTCACCTGCA
AAATGGGCATTATTTATGGTTCCCCCCAACCTTTGTTCATTTTCTTTTGCATTC
CTGATTATTGTGTGTAAAGAAAGTCTGTACATTGGGTTATAACACTAGATATTT
AAACTTACAGGCTTATTTGTAAACCATCATTTTAATGTCCTGTAATTAACATGG
TTATAACATGTACACTCCCCCCTACTCACCACACAACTTTTTTTGTGTGCGGTG
AAACCAATTTTGGTTTGCAATAAAATCTTGAAAACTATTTGCG

Fig. 12 (part 2 of 2)

MTVKTEAARSTLTYSRMRGMVAILIAFMKQRRMGLNDFIQKLANNSYACKHPEV
QSYLKISQPQEPELMNANPSPPPSPSQQINLGPSSNPHAKPSDFHFLKVIGKGS
FGKVLLARHKAEEAFYAVKVLQKKAILKKKEEKHIMSERNVLLKNVKHPFLVGL
HFSFQTADKLYFVLDYINGGELFYHLQRERCFLEPRARFYAAEIASALGYLHSL
NIVYRDLKPENILLDSQGHIVLTDFGLCKENIEHNGTTSTFCGTPEYLAPEVLH
KQPYDRTVDWWCLGAVLYEMLYGLPPFYSRNTAEMYDNILNKPLQLKPNITNSA
RHLLEGLLQKDRTKRLGAKDDFMEIKSHIFFSLINWDDLINKKITPPFNPNVSG
PSDLRHFDPEFTEEPVPSSIGRSPDSILVTASVKEAAEAFLGFSYAPPMDSFL

Fig. 13